THE CRANIAL AND SPINAL GANGLIA AND THE VISCERO-MOTOR ROOTS IN AMPHIOXUS.

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As my study of the central nervous system of *Amphioxus* must be interrupted for some months on account of other work, I will publish now a brief description of the cells of origin of the nerve components which constitute the dorsal roots.

Reviews of the literature on the nervous system of *Amphioxus* are given in papers cited below (nos. 3, 4, 5,) so that it will be necessary to speak here only of the work bearing directly on the elements to be described. Rohde (1) thought that the nuclei which he saw in the roots of the dorsal nerves indicated the presence of the equivalent of the spinal ganglia of vertebrates. On p. 199 he says: "Allenthalben liegen den sensiblen Nerven Kerne eingebettet, welche namentlich häufig bei ihrem Abgange vom Rückenmark auftreten. Sie haben genau dasselbe Aussehen wie die besonders in der Epithellage des Hirnventrikels häufig vorkommenden Nervenkerne und sind diesen sicherlich identisch, also nervöser Natur . . . Den Spinalganglien der höheren Wirbelthiere entspricht also bei *Amphioxus* eine Ansammlung nervöser Kerne." The author then reviews earlier comments on these nuclei. Neither Rohde nor earlier authors saw cell bodies or nerve processes belonging to them. Evidently the mass of nuclei described by Rohde is continuous with the small nests of ganglion cells mentioned by Hatschek (2). This author says: "Die dorsale Wurzel, welche bekanntlich keine Verbindung mit der ventralen eingeht, steigt nahezu in dem Winkel des Myoseptums gegen die Unterhaut empor und theilt sich dort in einen dorsalen und ventralen Ast. Kleine Nester von Ganglienzellen finden sich besonders an der Theilungsstelle des Nerven, z. T. aber auch schon in dem aufsteigenden Teile und auch in den Ästen. Der aufsteigene Teil ist daher als eine ausgezogene Wurzel zu betrachten und die Spinalganglien, welche wenig konzentriert sind, liegen in der Unterhaut (in unmittelbar Nahe

ihres epithelialen Entstehungsortes)." The methods used by these authors were inadequate to demonstrate the character of the cells to which they called attention and later authors working by special methods have denied the nervous nature of these cells.

Retzius (3) describes two types of cells within the spinal cord of *Amphioxus* which send fibers out in the dorsal roots. One type consists of small and medium sized bipolar cells transversely placed at either side of the central canal or extending across it. From one end of the cell a fiber enters the dorsal root. The second type consists of longitudinally placed bipolar cells from either end of which a fiber passes rostrad or caudad in the dorsal fiber bundles. One of the fibers arising from such a cell divides in T-form, sending a lateral branch into the dorsal root. After reviewing the relations of these cells Retzius says (p. 45): "Wo sind nun die *Spinalganglien*? Es giebt deren *keine*. In den sensiblen Wurzeln sucht man sie vergebens, sowohl innerhalb der Rückenmarksgrenze wie ausserhalb derselben. — In dem nächsten Verlauf der sensiblen Zweige trifft man weder einzellne Ganglienzellen noch Gruppe von solchen. Die Asnchwellungen, welche einige Autoren erwähnen, waren gewiss nur zufällige Bildungen. Ebensowenig konnte ich etwaige Stellvertreter, sog. 'Analoga,' der Spinalganglien entdecken; auch die von Rohde in die Wurzeln eingebetteten Kerne, die er geneigt ist als solche zu betrachten, sind meiner Ansicht nach nicht nervös, nicht 'Analoga' der Spinalganglien.

"Wenn also 'Analoga' oder richtiger Homologa der Spinalganglienzellen ausserhalb des Rückenmarks nicht nachweisbar sind, so bleibt die Frage unbeantwortet, ob nicht entsprechende Ganglienzellen im Innern des Rückenmarks vorkommen. Und man hat dann daran zu denken, ob nicht die beiden Reihen longitudinal angeordneter Ganglienzellen, deren Stammfortsätze nach geschehener Teilung (in T-form) in die sensiblen Wurzeln austreten, möglicherweise den Ganglienzellen der Spinalganglien entsprechen können."

Heymans and van der Stricht (4) described the development of the dorsal root by two rootlets, a dorsal cellular and a dorsolateral fibrous strand. With regard to spinal ganglia they say

(p. 16): "Si l'on examine très attentivement des diverses parties constituantes, aux différents stades de leur développment, de cette double ébauche radiculaire, nulle parte on ne constate une trace d'ébauche ganglionaire. L'étude de l'Amphioxus adulte, à l'aide de la méthode de Golgi, à l'aide du bleu de méthylène et à l'aide des méthodes ordinaires, ne révèle l'existence d'aucune cellule ganglionnaire proprement dite sur le trajet des racines dorsales, mais dévoile leur présence à l'intérieur du névraxe lui-même. Nous croyons donc pouvoir en conclure qu'il n'existe point de ganglions spinaux ni de ganglions crâniens sur le parcours des racines dorsales chez l'Amphioxus."

Dogiel (5) has described at length certain structures which he finds attached to the nerve rami just distally from the point of division of the dorsal roots into dorsal and ventral rami. The structures appeared in animals subjected for a long time to methylene blue in physiological salt solution. They were never stained by methylene blue dissolved in sea water. Dogiel was uncertain whether they may not have been artificial products due to immersing the animals in physiological salt solution. He was unable to make out their structure in preparations fixed and stained in various ways. He decided that they are normal structures, however, since they stain well with gold chloride and are visible in animals treated with osmic acid. The structures in question are rounded or pear-shaped bodies which are found in clusters of two, three or four attached to the nerve rami as berries are attached by their stems. The author concludes: "Sämtliche aufgezählten Thatsachen lassen sich am ehesten in der Weise auslegen, dass die beschriebenen Elemente Analoga von Spinalganglien darstellen, welche beim Amphioxus möglicherweise in einer embryonalen Entwicklungsform vorhanden sind. Eine endgiltige Entscheidung der Frage über die Natur dieser Gebilde ist jedoch natürlich nur dann möglich, wenn die Structur derselben und ihre Beziehung zu den Nerven sicherer bestimmt sein wird."

I can add with regard to these structures, first that they do stain by methylene blue dissolved in sea-water, and second that they are to be found along the course of the ventral ramus as far ventrally as the middle of the lateral surface of the body.

Although I have had them stained in a large number of animals they have appeared only in specimens which had remained a long time in a relatively strong stain or had been exposed to the air for a long time after staining, or both. In all cases the animals, although alive and reflexly irritable, were in an extreme state of weakness. I have never seen in these bodies stained with methylene-blue such a structure as would indicate that they are normal nervous organs. They always appear as pale, granular or structureless pear-shaped or balloon-shaped bodies attached by the small end to the nerve ramus. I do not see that any fact regarding these bodies suggests comparison with the spinal ganglia of vertebrates. Their form, their position, the fact that they appear stained with methylene-blue only when the animals are subjected to physiological salt solution or are kept in stain in sea water until they have reached a state of extreme weakness, all indicate that they are probably artifacts. From my own observations I should conclude that they are formed by the exudation of fluid from the nerve through a rupture in its sheath. The exuded fluid takes the form of a balloon, remaining attached by a stalk. The most favorable place for the formation of such exudation is in the angle between the dorsal and ventral rami and between two myotomes. They are formed also, but less often, far along the ventral ramus and even relatively far out along the dorsal ramus. If by physiological salt solution in which Dogiel stained his animals, he means a solution of sodium chloride of 0.75 per cent., more or less, this would be very favorable to the formation of such artifacts, since a physiological salt solution *for Amphioxus* must contain upwards of three per cent. of sodium chloride. The animals soon die in salt solutions of less strength. Dogiel states indeed that his animals died quickly in the physiological salt solution and that the structures described were brought to view by the stain only after three or four hours! Maceration must have been going on rapidly during all that time.

Finally, I have been unable to find these bodies in sections of animals well fixed and stained by various methods, or in specimens treated with osmic acid. The resemblance which Dogiel notes between these bodies and certain structures connected with the rostral nerves is not at all close. On the proximal portion

of the first, second, third and fourth nerves of normal living animals are to be seen rounded projections or knobs which might be described as bud-like or as resembling an up-turned thumb. The greatest difference between these structures and the supposed spinal ganglia is that these stain in methylene-blue at the same time with those bodies which are connected with the end branches of the rostral nerves; that is, some hours earlier than the supposed spinal ganglia.

My own observations have been made upon animals stained in methylene-blue and upon sections prepared by the Golgi method and by a variety of hæmatoxylin methods after fixation in twenty per cent. formalin, Worcester's, Zenker's or Flemming's fluid.

In living animals stained with methylene-blue, both types of cells described by Retzius and the fibers from the transverse cells passing into the dorsal roots have been clearly and repeatedly seen. In two specimens also, many examples of slender bipolar cells were seen in the root of the nerve in the situation of the cells shown in Fig. 4. Each of these cells sent one process out along the nerve and one into the cord. When the cells in this position are examined in hæmatoxylin sections they are seen to be spindle-shaped cells with elongated nuclei and with a process from each end of the cell which can be followed for a considerable distance. The cells have very slender bodies with only a thin layer of cytoplasm over the nucleus. Except for the difference in size, which is more or less proportional to the difference in the size of the two animals, these cells are closely similar to the ganglion cells of the cutaneous and lateral line fibers in Petromyzon (*Lampetra Wilderi*, 6). Such cells are found in the root and undivided trunk of the nerve, in the proximal part of the dorsal and ventral rami, and also in the cord near the root of each nerve. The largest number of cells are found in the proximal part of the root and in the somewhat conical protuberance of the cord which gives rise to the root. In the so-called cranial nerves (I., II.) the large size of the roots and the somewhat more regular arrangement of the cells and fibers enable one to see these elements more clearly than in the trunk nerves. Comparison of horizontal and transverse sections shows that in both cranial and trunk nerves the cells are distributed throughout the thickness of the nerve root.

Sections by the rapid Golgi method show these cells and confirm the description given above. Cells of the following types are seen to send fibers into the dorsal roots. In the figures the several types are indicated by the arabic numerals which are used here in the text. (1) Bipolar cells near to or extending across the middle line of the cord, whose central processes go to the further side of the cord from the roots which the peripheral processes enter. The central process after reaching the opposite side of the cord either (*a*) enters the dorsal bundles without dividing, (*b*) divides in T- or Y-form into rostral and caudal branches, or (*c*) ramifies at once more or less profusely. The last mode of behavior is seen most often in my preparations. These cells mingle with the more centrally situated cells of the next type. (2) Bipolar cells within the cord more or less radially placed with reference to the nerve root, whose central processes remain on the same side of the cord as the roots which the peripheral processes enter. These cells may be situated anywhere within the area of a fan whose handle is represented by the nerve root and whose rays are represented by these cells and their processes. The bodies of the cells are situated among the bundles of root fibers as they turn for-

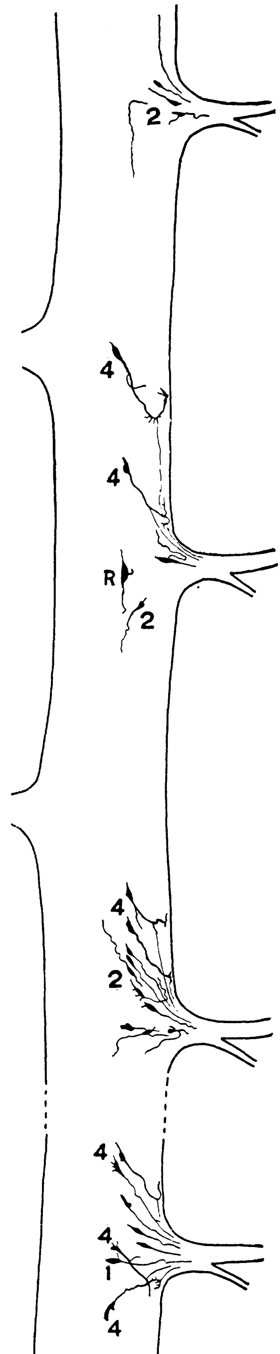

FIG. 1. A horizontal section of the nerve cord and dorsal roots. The parts above and below the dotted lines were drawn from adjacent sections of the same animal. The arabic numerals indicate the types of cells described in the text. *R*, longitudinal bipolar cell of Retzius. The smaller dorsal ramus of each nerve diverges caudally from the larger ventral ramus.

ward or backward in the cord, and the central processes run with the root fibers in the dorsal bundles of the same side. More cells of this type are impregnated than of either of the others but only enough have been drawn in the figures to illu-

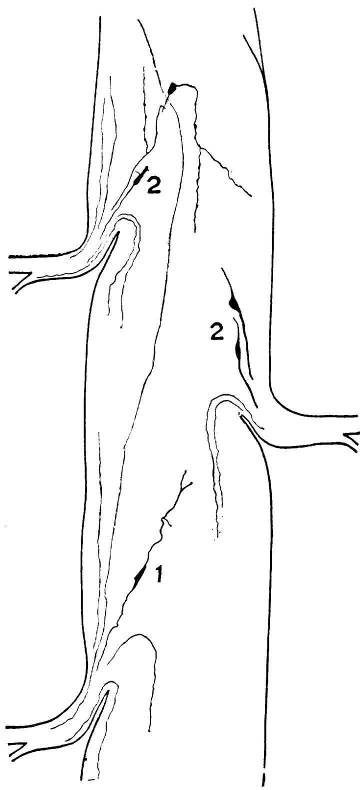

FIG. 2. Horizontal section of the cord between the tenth and eleventh dorsal nerves. The form of the nerve roots shown is characteristic of this region of the body. On the right side are two very coarse fibers with their ganglion cells.

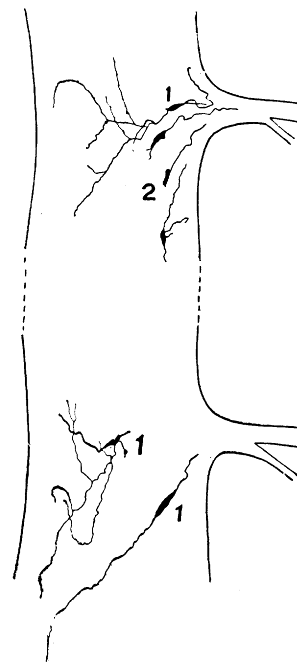

FIG. 3. Horizontal section showing especially fibers going to the opposite side of the cord.

strate their position. (3) Bipolar cells in the root or trunk of the nerve whose central processes are seldom impregnated far into the cord. Those that are impregnated enter the dorsal bundles of the same side. Many root fibers are impregnated which show no cells connected with them. These all run forward or backward in the dorsal bundles of the same side. They

are doubtless fibers whose cells are situated in the root or trunk of the nerve. As shown in Fig. 4, these cells are sometimes present in the dorsal and ventral rami, and it seems probable that they will always be found there. I have not been so fortunate as to have any cells impregnated far out beneath the epidermis in the position indicated by Hatschek, although such cells are readily seen in hæmatoxylin sections. (4) Irregularly pyramidal cells situated near the canal at a slightly more dorsal level than the pigment cells. These cells are usually provided with a single coarse process which runs to the surface of the cord where it ends in a few thick branches or in broad plate-like expansions against the limiting membrane. From some point in its course this thick process (dendrite) gives off a fine fiber which enters the dorsal root. Eight of these cells are shown in Fig. 1.

There can scarcely be any question of the homology of the cells of the first three types described with the spinal ganglion cells of vertebrates. The description confirms the account given by Retzius of cells within the cord sending fibers into the dorsal roots, but it shows that by far the larger number of such cells are situated where Retzius distinctly denied the existence of any nerve cells. They are the cells whose nuclei attracted the attention of Rohde and the earlier authors. The facts given by Retzius together with the discovery (7, 8) that the giant cells in the cord of teleosts are comparable with spinal ganglion cells have been considered as evidence that the spinal ganglia in vertebrates have been derived from the spinal cord. Now that the disposition of the ganglion cells in *Amphioxus* is more fully known it shows that this animal is not so different from vertebrates in this regard as was supposed. In *Amphioxus* the spinal and cranial ganglia form for each nerve an almost continuous mass extending from the central canal of the cord out into the root of the nerve to and beyond the division into dorsal and ventral rami. Thus it may be said that part of the ganglion cells in *Amphioxus* occupy a place within the cord which has been regarded as the hypothetically primitive position for vertebrates, while most of them occupy a place in the nerve roots which approaches the typical position for higher vertebrates. *Amphioxus* is, therefore, not quite primitive in this matter but rather approaches typical vertebrates.

One fact makes it seem probable that there is a movement peripherally of the spinal ganglion cells in *Amphioxus* after the period in the ontogeny when the longitudinal fibers of the cord are formed. Many of the fibers adjacent to the roots are bent out far into the root where they recurve and pass on in their former course within the cord. A slight case of this is shown in Fig. 4. In many cases such recurved fibers extend much further out into the nerve roots, and the number of fibers affected in this way is so great that the bulging laterally of the cord toward each root is very striking in horizontal sections. Occasionally some of the giant fibers are carried toward a root until

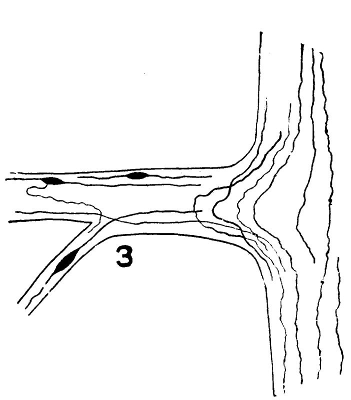

FIG. 4. Horizontal section of dorsal root showing ganglion cells in the trunk and rami. Combined from two sections.

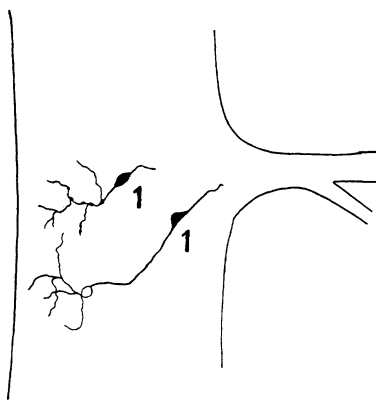

FIG. 5. Two cells whose neurites ramify at once on the opposite side of the cord.

they seem as if they were about to enter it. I can think of no other cause for this curving of fibers out into the roots except the possible active migration of the ganglion cells.

The place of branching of the dorsal roots into dorsal and ventral rami is of some interest. The branching seldom takes place close beneath the dermis as Hatschek describes it. On the other hand, the division of the root near the cord and the separate origin of dorsal and ventral rami directly from the cord are of more frequent occurrence than Rohde states, and are not confined to the anterior end of the body. The separate origin of the rami may be seen in the case of one or several roots in a considerable majority of specimens. Every possible gradation

is to be found between this and the manner of division described by Hatschek. The typical place of division is about half way between the cord and the dermis. This is not only the mean between two extremes, but it is the place where the rami separate in the great majority of cases. In proportion as the division into rami occurs nearer the cord, a greater number of ganglion cells are found in the rami. The argument of Fürbringer (9, p. 646) that the lateral musculature of *Amphioxus* corresponds to the mesial portion only of that of craniates, based upon Hatschek's description of the spinal ganglia of *Amphioxus* outside the muscles, is not supported. The greater part of the spinal ganglion of *Amphioxus* is situated mesial to the muscles as in craniates.

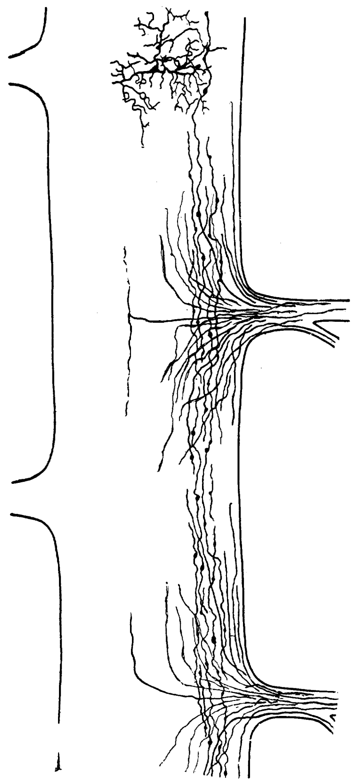

FIG. 6. Horizontal section showing the spreading of the sensory roots into mesial fine-fibered, lateral coarse fibered bundles and the formation of the dorsal compact bundle. The fibers of the last bundle are strongly varicose. At the upper part of the figure is shown the end-branching of a coarse sensory fiber from the root opposite.

It is hoped that the cutaneous and visceral sensory fibers in the dorsal roots may be distinguished, the position of their respective ganglion cells determined, and the central course of each component traced. The more general facts regarding these points seem now to be clear.

The great majority of the fibers of the dorsal roots are fine or medium sized ; a few only are very coarse. The disposition of the fibers in the cord can be seen best in horizontal sections prepared by the Golgi method. As they enter the cord the fibers spread forward and backward and many pass to or across the

middle line, diverging more or less. The fibers which remain on the same side separate into two ill-defined bundles, of which the one nearer the median line consists of the fibers. The medium and coarse fibers are situated laterally. In a high focus, somewhat above the root of the nerve, a distinct bundle of fibers is seen running along dorsal to and independent from the spreading fibers of each nerve. Some fibers of each nerve, however, enter this bundle. The bundle consists of medium coarse fibers

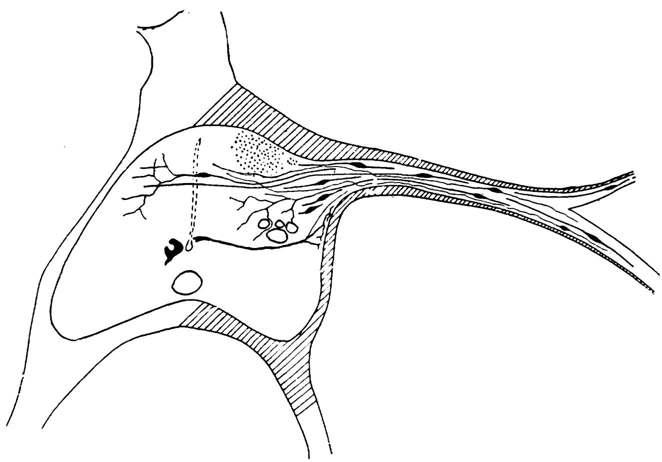

FIG. 7. A diagrammatic transverse section of the nerve cord and a dorsal root. The sheath of the nerve cord is marked with oblique lines. The right lateral group of giant fibers, the mid-ventral fiber and one pigment cell are shown. The stippled area shows the position and size of the compact dorsal bundle of fibers as it appears in the middle region of the body. The disposition of the coarse and fine fibers and their ganglion cells and the position of one viscero-motor cell are shown.

and is situated at the surface of the cord between the mid-dorsal line and the nerve roots. Its position is shown in Figs. 6 and 7. This bundle is distinctly seen in hæmatoxylin sections but has a very different appearance after different fixing agents. It is not well fixed in all fluids. In Zenker's fluid it contracts and the fibers become aggregated into a dense mass which is surrounded by an open space. In twenty per cent. formol the bundle has the appearance of poor fixation with swelling. It appears as a lightly stained reticulated area in which the fibers are not sharply visible. In both these fluids the remainder of the cord seems to be well fixed and stains well. In Worcester's fluid, which has

remarkable penetrating qualities and is a faithful fixing agent, there is no shrinking or swelling and the fibers are well fixed. The bundle appears as an area of rather coarse fibers which take a deeper stain than the remaining fibers in the dorsal region of the cord. The bundle extends throughout the whole length of the nerve cord, at least from nerve II. well into the tail region where it grows very small. The bundle is very noticeably larger on the right side than on the left and on both sides the bundles increase in size toward the head end, perhaps because the majority of the fibers are ascending. In the head region the bundle of the right side is augmented considerably by fibers from each of the nerves VI.–III. inclusive. These fibers run forward and mostly leave the bundle within one segment, for the nerve is small immediately behind each of the nerves mentioned. In brief, the sensory roots form three tracts in the dorsal part of the cord, a diffuse mesial tract of fine fibers, a diffuse lateral tract of coarser fibers and a compact dorsal superficial tract of coarse fibers. The first and second mingle more or less with one another and with fibers of other kinds running ventral to them. The ventral limit of the dorsal tracts taken as a whole is roughly marked by the lateral group of giant fibers.

The very fine fibers seem never to be connected with ganglion cells within the cord, but some of them do have their cells in the nerve trunk. They run for a comparatively long distance in the cord without dividing. Since the visceral sensory fibers in vertebrates are fine, the hypothesis presents itself that these are the visceral fibers in *Amphioxus*. With this their position near the dorsal raphe is consistent. Evidently the visceral fibers do not enter the well defined bundle of coarser fibers ; for, if they did, that bundle should be very large on the left side in the head region where all the visceral surface is supplied by nerves of the left side. The bundle is small on the left and larger on the right.

The coarser fibers comport themselves in a variety of ways on entering the cord. (In speaking of coarse and fine fibers of the dorsal roots one must compare the central processes of ganglion cells with central processes and peripheral processes with peripheral processes and in order to do this one must know where the ganglion cell of a given fiber is located.) Speaking then of

the coarser central fibers, some of them ramify at once on entering the cord, others divide in T-form and run in the dorsal bundles of the same side, others enter the dorsal bundles without dividing, others go to the opposite side of the cord and either ramify at once or run in the dorsal bundles, with or without bifurcation. Altogether, the number of coarser fibers which ramify near to the root from which they come is striking. If these are cutaneous fibers a striking physiological fact is explained, namely the relative independence of the segments in locomotion; otherwise expressed, the small number of segments necessary to perform the typical swimming movements. A short piece of the tail end can swim well and behaves much as a whole animal does; and this for many days together.

To illustrate by a complex movement, normal animals in a shallow dish persistently put their heads up over the edge of the dish and then by swimming round the dish and pushing against the edge succeed in wriggling over, if the edge is not too high. The isolated tail makes the same persistent and apparently purposeful efforts when the dish is very nearly full of water. When an animal is so macerated that *all* the tissues except the notochord are gone from the middle of the body, the two parts perform typical swimming movements but each with an independent rhythm. This retention of the power of coördinated movements by a few isolated segments is perhaps connected with the large number of cutaneous fibers which have a short course in the spinal cord. This makes it possible for the muscles to be reflexly controlled by stimuli received at the surface of the body in the same or adjacent segments.

Finally, a few coarse fibers whose ganglion cells are in the nerve trunk go to form the definite bundles described above. Since these are chiefly ascending fibers which have a long course in the cord, the bundle may be compared with the dorsal tract of the same description in vertebrates, the tract of Goll. This is therefore probably the first tract to appear as a definite bundle in the vertebrate nervous system.

The fourth type of cells described in this paper are the viscero-motor cells. In position they correspond to the visceromotor column as it is known in fishes and other vertebrates.

They are lateral to the ventral parts of the canal. They retain primitive characters in that the cell body is adjacent to the canal and that there is a single large dendrite which extends to the periphery of the cord. The origin of the neurite from the dendrite at some distance from the cell body is perhaps also a primitive character so far as the vertebrate nervous system is concerned. With respect to the disposition of these cells along the cord it is evident that they form a more or less complete column and that the neurites often run for some distance in the lateral tracts to reach their roots (Fig. 7).

As our knowledge of the nervous system of *Amphioxus* increases its effect is to oppose the tendency of recent years to minimize the relationship between *Amphioxus* and vertebrates, to consider *Amphioxus* as far removed from typical vertebrates and more closely related to invertebrates rather low in the scale. While there is great significance in the similarity of the nephridium (10) and eye-spots (11) of *Amphioxus* to those of some worms, the close relation of the nervous system of *Amphioxus* to that of vertebrates has also an unquestionable significance. *Amphioxus*, indeed, contributes more toward bridging over the gap between vertebrates and invertebrates than has usually been supposed.

The nervous system of *Amphioxus* agrees with that of lower fishes in the following respects:

(*a*) It is dorsal, hollow, and has separate dorsal and ventral roots of definite composition. The canal has an enlargement at the anterior end, the brain ventricle.

(*b*) The dorsal roots consist of general cutaneous, visceral sensory and visceral motor components. They contain also in the head region fibers of special sense organs (olfactory or gustatory ?).

(*c*) Both kinds of sensory fibers have ganglion cells which are situated either within the cord or in the root of the nerve in essentially the same position as the spinal ganglia of vertebrates.

(*d*) The two kinds of sensory fibers on entering the cord form dorsal tracts similar to those in vertebrates. Many cutaneous fibers show the bifurcation characteristic of these fibers in vertebrates.

(*e*) The viscero-motor cells are situated as in vertebrates dorsal to the somatic motor cells, lateral to the ventral part of the canal.

(*f*) The nerve cells retain the position and characters which are typical in the embryos of vertebrates and which are seen in certain parts of the brain of many fishes.

(*g*) The ventral roots arise separately and remain independent. They are true somatic motor nerves.

We have here nothing else than an essentially vertebrate type of nervous system. At the same time there are good indications of a truly primitive, unspecialized condition. In addition to the facts given under *a*, *e* and *f*, there may be mentioned:

(*h*) The total absence of certain specialized structures which characterize all vertebrates, namely, hair cells responding to vibrations in fluid (neuromasts or acustico-lateral organs), and retinal visual organs. (The morphology of olfactory and gustatory sense organs is yet in an uncertain state.)

(*i*) The presence of simple light-percipient organs within the central nervous system, which have apparently been retained from the worm-like ancestors of *Amphioxus*.

(*j*) The very slight development of the brain.

These facts speak eloquently against the supposition that *Amphioxus* is the result of a process of degeneration from any form which had reached a higher degree of specialization, such as selachians or other fishes. Similar facts have been brought forward (6, p. 73) to show the primitive character of the cyclostome brain. We cannot suppose that specialized structures in the nervous system once possessed by the ancestors of *Amphioxus* have been nicely pruned back and reduced in the process of degeneration; nor can we believe that true nephridia and helminthine eye-spots should reappear in a degenerated species whose ancestors in the course of their evolution had lost these very organs. The straightforward interpretation of the nervous system supports the view that *Amphioxus* and Cyclostomes are the lower branches of the vertebrate phylum. [1]

[1] The Worcester's fluid spoken of in the text is as follows:

40 per cent. formalin	10 parts,
Distilled water	90 "

Saturate this with sublimate;
Add glacial acetic to make 10 per cent.

This work has been done at the Smithsonian table in the Naples Zoölogical Station. I wish to acknowledge my great obligation to the Institution for the opportunities thus afforded, and to express my best thanks to the officials of the zoölogical station for the excellent facilities provided.

NAPLES, January 25, 1905.

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